ELECTROPORATION OF LIGNOCELLULOSIC BIOMASS

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Bachelor of Engineering Technology (Electrical) With Honors

UNIVERSITI MALAYSIA PAHANG

STATEMENT OF AWARD FOR DEGREE

1. Bachelor of Engineering Technology Final Year Project Report

Thesis submitted in fulfilment of the requirements for the award of the degree of Bachelor of Engineering Technology in Electrical with Hons.

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Engineering Technology in Electrical with Hons.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the guidance and the help of several individuals who contributed and extended their valuable assistance in the preparation and the completion of this study. Foremost, I would like to take this opportunity to express my sincere and deepest gratitude to my supervisor, Assoc. Prof. DR Che Ku Mohammad Faizal for the unlimited support, patience, motivation, guidance and knowledge for completing my thesis. Throughout the process of doing this senior design project (SDP), I have gained a lot of knowledge, skills and expertise such as time management skill, work together with project members inside the workshop, communication skill and so on. It is really an honour for me to work under his supervision as he has offered me his precious time and efforts in completing this SDP.

Apart from my supervisor, my sincere thanks to Professor Dato' Dr. Zularisam Ab Wahid, Dean of the Faculty of Engineering Technology, for providing me with all the necessary facilities and financial budget to complete the whole senior design project. My sincere thanks also go to the rest of the staffs from Faculty of Engineering Technology (FTEK) in University Malaysia Pahang (UMP), all the lecturers and JP's for helping and guiding me in the workshop for the circuit designs, product building, experimental analysis and data collection. Their kindness and patience offering helps are very much appreciated.

Throughout this whole project, I established good teamwork with my groupmates, Noor Atikah Binti Buang and Nor Nadhirah Binti Abdullah, and I would also like to take this opportunity to thank them for the great teamwork in order to finish this project as a whole.

Last but not least, I would like to express my utmost, sincere gratitude to both my parents and family members for all those motivations and encouragements throughout my studies, especially for the completion of senior design project. It's been a wonderful learning experience while working on this project.

ABSTRACT

Electroporation (EP) is versatility, rapid and high efficiency pretreatment technique using high voltage toward biomass feedstock. It is effectively and applicability to nearly all cell and species types. At the same time, Lignocellulosic biomass (*Pistia Stratiotes*) is the most abundant renewable bioresources on earth. The cellulose and hemicellulose contained in lignocellulosic biomass can be suitable substrate for bioethanol production. However, electroporation pretreatment technique still not widely use in this world. Therefore, this project was designing and fabricating an electric circuit and reactor to conduct electroporation treatment on lignocellulosic biomass (*Pistia*) in order to promote electroporation pretreatment technique. The performance of the circuit over the cell structure changes of the *pistia* will then test by doing electroporation treatment. In this project, Bluetooth HC-05 module was use as a region to communicate mobile phone with Arduino to control pulse width modulation (PWM) to produce high voltage square wave pulse. The high output voltage was produced by a DC step-up pulse generator which draw input source from 6V battery and controlled by SPDT relay for switching ON/OFF purpose. To prioritize the safety of user, LCD, buzzer and LEDs added to give signal and warning to user when the electric circuit is working. Bluetooth also one of the safety feature because user can control circuit by mobile phone and do not have to contact with the circuit to reduce the chance for getting shock. The reactor contains the container and electrodes with use to put the biomass sample and conduct treatment. Acrylic is chosen as the material to fabricate the container due to its transparency, electric and thermal insulated. Aluminum sheet was cut into small size to use as electrodes because it has high conductivity, not easy to get rust and low cost. Electroporation treatment is carried out to validate the electric circuit and reactor. The leaves of *pistia stratiotes* was dry in oven and then mashed into small particle to mix in water for treatment purpose. Sample was collected before treatment and during treatment from the mixture and then the sample cell structure analyzed under scale electron microscope (SEM). As a result, very obvious crack can be observed on the sample cell structure that undergo electroporation treatment compare to the sample cell before treatment. From the result, the cell structure of *pistia* was successfully disrupted, cellulose and hemicellulose can be extracted from it.

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LIST OF SYMBOLS

V	Volt
S	Second
min	Minute
SEM	Scale Electron Microscope
EP	Electroporation
IRE	Irreversible Electroporation
DNA	Deoxyribonucleic acid
kV	Kilo Volt
Ω	Ohm
Hz	Hertz
ms	Milli second

LIST OF ABBREVIATIONS

IDE	Arduino Software
I/O	Input / Output
SPDT	Single Pole Double Throw
DC	Direct Current
SPP	Serial Port Protocol
PWM	Pulse Width Modulation

CHAPTER 1

INTRODUCTION

In this chapter, the background and the problem statement are determined. Objectives and scope of study are identified to achieve the project purpose and overcome the problem statement.

1.1 BACKGROUD OF STUDY

Electroporation was initially developed for the introduction of DNA into cells which grow in suspension and was performed in a cuvette with two flat electrodes on opposite sides. Different configurations were subsequently developed for the electroporation of adherent cells in situ, while the cells were growing on nonconductive surfaces or a gold-coated, conductive support. We developed an assembly where the cells grow and are electroporated on optically transparent, electrically conductive indium-tin oxide (ITO). This material promotes excellent cell adhesion and growth, is inert and durable, and does not display spontaneous fluorescence, making the examination of the electroporated cells by fluorescence microscopy possible. The molecules to be electroporated are added to the cells and introduced through an electrical pulse delivered by an electrode placed on top of the cells.

Electroporation or electropermeabilization is a transformation technique that uses induction of macromolecular uptake by exposing cell walls to high-intensity electrical field pulses. The effectiveness of microalgal electroporation was first reported by Brown et al., 1991. Electroporation specifically disrupts lipid bilayers, leading to efficient molecular transport across the plasma membrane. Efficient electroporation-mediated transformation was achieved in both wild-type and cell wall–deficient Chlamydomonas cells (Brown et al., 1991). The transformation efficiency of electroporation is two orders of magnitude higher than the glass beads method, and only requires relatively simple equipment. Important parameters affecting the effectiveness of electroporation include field strength, pulse length, medium composition, temperature, and membrane characteristics, as well as the concentration of DNA (Wang et al., 2007).

Our project focuses on the mainly in irreversible electroporation in which the structure of the cellulose or hemicellulose of lignocellulosic biomass will be disordered or deformed to release fermentable sugar. Irreversible electroporation (IRE or NTIRE for non-thermal irreversible electroporation) is a soft tissue ablation technique using ultra short but strong electrical fields to create permanent and hence lethal nanopores in the cell membrane, to disrupt the cellular homeostasis. The resulting cell death results from apoptosis and not necrosis as in all other thermal or radiation based ablation techniques.

1.2 PROBLEM STATEMENT

There are a lot of pretreatment methods on biomass feedstocks had already investigated. The alternative pretreatment of biomass feedstocks include ultrasound, microwave, extrusion, etc. Lignocellulosic biomass is one of the complicated pretreatment as the main obstacle for commercial use. Lignocellulosic feedstock materials are the most abundant renewable bioresource material available on earth. We are trying to make this pretreatment to be success and can be used more widely in biomass pretreatment so we are doing electroporation of lignocellulosic of biomass. We decided to use electroporation (EP) as the pretreatment to lignocellulosic biomass because electroporation is versatility. It is effectively and applicability to nearly all cell and species types. EP is easy, rapid and high efficiency. It able to transfect a large number of cells within shorter time when higher voltage is given. We are using Irreversible Electroporation (IRE) as the method to conduct the treatment on lignocellulosic of water lettuce (*Pistia Stratiotes*). IRE can fully damage the structure of the cell and cause the cell totally disrupted by high voltage pulse and only partially of the membrane can be repaired. Bioethanol can be produced by additional steps apply to the liquid that take from the structure of *Pistia* after EP treatment. The electroporation machine which available on market is not so convenient to carry and need to control by human themselves with contacting with the equipment, it may unsafe for user. From here, we are trying to solve this problem and come out the objective of fabricate a Bluetooth based electric circuit and reactor to conduct electroporation. The portable smaller size of the device might easy for user to carry. As required, high voltage square wave pulse must be created for electroporation pretreatment technique.

Bioethanol is a renewable resource and also the most useful solution to all the issues related to environment and energy crisis. Bioethanol is biodegradable and much less toxic than fossil fuels. Air quality can be improved by using bioethanol in older engines by reduce the amount of carbon monoxide produced by the vehicle. To promote Bioethanol production and its uses, we reduce the production cost by using cheap substrate which is lignocellulosic *Pistia* and also to obtain the suitable microorganisms which provide sufficient fermentation yield. *Pistia* consisting 27.55% of cellulose and 29.71% of hemicellulose can be suitable substrate for bioethanol production by providing pre-treatment to *Pistia* (Sivasankari. B, David Ravindran. A, 2016).

Lignocellulosic of *Pistia* is the most abundant resources, low cost pre-treatment skill and less harmful to environment, while electroporation is the most suitable pre-treatment skill to lignocellulosic biomass. So we suggest that electroporation of lignocellulosic biomass can be one of the most effective and useful skill to produce bioethanol and can be used widely in the future.

1.3 OBJECTIVE

1. To design a Bluetooth based electric circuit to produce high voltage square wave pulse.

2. To fabricate a reactor to conduct electroporation.

3. To validate the performance of circuit over the structure changes of the lignocellulosic biomass (*Pistia Stratiotes*).

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